THE DISTRIBUTION OF SOIL BACTERIA IN RELATION TO BIOLOGICAL ACTIVITY AND PEDOGENESIS

PART 1. GENERAL INTRODUCTION AND FACTORS AFFECTING POPULATIONS AT TAITA EXPERIMENTAL STATION, NEW ZEALAND

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Summary

Differences in numbers and kinds of bacteria at Taita Experimental Station were determined from plate counts of pasteurised and unpasteurised soil dilutions and by growth in glucose broth. Differences in bacterial populations are discussed with regard to sampling and seasonal variation, and to differences in vegetation, particularly between a site under beech forest and one under grassland. Differences were found not only in numbers of the main groups but also in the distribution of particular species such as *Pseudomonas fluorescens* which occurs in detectable numbers only in moist grassland conditions. *Bacillus megaterium* was the most common aerobic spore-forming bacterium isolated from both sites but the *B. circulans* group was also common. Changes in population following change of vegetation are discussed with regard to possible colonisation by airborne flora.

Warburg respiration measurements made in the laboratory suggest that inadequate moisture limits biological activity only in the beech forest litter, but not in the beech topsoil or in the grassland topsoil. Forest litter showed the greatest increase in activity when glucose was added. Beech topsoil samples tend to have higher respiratory rates than grassland topsoil samples, and this is correlated with the greater addition of organic matter to the soil under forest.

GENERAL INTRODUCTION

Two associated processes transform the mineral mantle of the world's land surface into soil: weathering and the organic cycle. Weathering comminutes the mineral parent material into particles of decreasing size (sand to silt, silt to clay), converts colloids to crystalline clays and leaches out, retains, or redistributes through the profile the products of physical and chemical decay. The organic cycle transforms solar energy into chemical energy and mobilises mineral nutrients to provide a dynamic interchange between soil and plant. It effects, in addition, a heightened rate of physical and chemical change through the cycle of plant growth and decay.

The microflora, particularly the bacterial flora, is intimately associated

with both these processes. As pedogenesis proceeds, therefore, one might expect changes in the bacterial flora to be associated not only with changes in the soil properties, but also with changes in the soil processes. For this reason, the present study of the bacterial flora of a range of soils throughout the region of the South Pacific Basin has been orientated to answer two questions:

(a) What differences are there in the bacterial flora of widely differing soils?, and
(b) How are they related to the soil processes taking place within the broad climatic zones of this area?

Three criteria have been used to answer these questions: difference in numbers; difference in kinds; and difference in activity.

Few groups of soil bacteria are taxonomically well defined. Of the aerobic heterotrophic bacteria, well described groups include the aerobic spore-forming *Bacillus* species (Smith *et al.*, 1952), the aerobic pseudomonads, i.e. *Pseudomonas fluorescens* and related taxa (Stanier *et al.*, 1966), *Chromobacterium lividum* (Sneath, 1960), and *Enterobacter* (*Aerobacter*) *cloacae* (Edwards and Fife, 1955), whose distribution in soils of this area has been previously studied (Stout, 1958; 1960a, b; 1961a, b; 1964). The survey examined the distribution of these few well-described species, and the levels of populations likely to indicate broad microbiological differences between soils. These population levels were determined by a total bacterial plate count; a count of bacterial spores and aerogenic fermenters; an estimate of the incidence of the epiphytic bacterial flora, typically yellow or orange chromogens—which often infiltrate the topsoil; and an estimate of the actinomycetes and moulds, which are the two significant antibiotic-producing microbial populations (Stout, 1961b).

Bacterial numbers are also affected by the activity of micropredators, particularly protozoa. No attempt has been made in the present papers to deal with this aspect of their ecology.

THE SOILS SAMPLED AND THEIR ENVIRONMENT

With few exceptions the soils sampled in this study lie in the South Pacific between longitudes 160°E and 160°W. The area covered extends from the equator to 86°S, and includes:

(a) the tropical islands of Hawaii, Fiji, Niue, Samoa, and the Cook Islands;
(b) the sub-tropical island of Raoul in the Kermadec group;
(c) the main islands of New Zealand, which are in the temperate zone;
(d) the sub-antarctic islands: The Snares islands, Auckland Island and Macquarie Island, which are in the sub-polar zone; and
(e) the Ross Dependency in Antarctica, in the polar zone.

Soil Parent Materials

The region is one of great tectonic activity and includes many volcanic islands. Consequently, the parent materials of many of the soils tend to be of geologically recent origin, and the topography offers sharp contrasts of relief and aspect. Many of the soils of the tropical and sub-tropical islands, and of the northern part of North Island, New Zealand, are derived from igneous rocks. Outcrops of coral limestone in the islands are a common parent material, and many of the soils of New Zealand are derived from indurated sediments and from metamorphic rocks, either by rock weathering *in situ* or by deposition of loess or alluvium. Most other New Zealand soils are derived from accumulating coastal sands or from peat. The sub-antarctic islands of New Zealand are generally covered with blanket peat overlying basalt or granite. The rock wastes of Antarctica are also derived from a variety of parent materials but accumulation of organic material is confined to lakes or exceptionally moist rock crevices.

Climate

The most extreme climatic conditions obtain in the continents, e.g. in Antarctica, which is a polar desert. The sub-antarctic islands have a cold temperate or sub-polar oceanic climate. The climate of the tropical and sub-tropical islands, and of New Zealand, ranges from sub-humid to super-humid, except for a small semi-arid area in the centre of South Island, New Zealand. The prevailing rain-bearing wind over the Pacific is westerly, and consequently, the rainfall gradient from west to east is as marked as is the gradient from low to high altitudes. At high altitudes in the tropics, e.g. in Samoa at an altitude of 5,000 ft (1640 m), rain carried by trade-winds strongly affects the climate. Rainfall in New Zealand is fairly evenly distributed, although some areas experience dry and wet seasons as do many of the Pacific islands. Except for those of Antarctica, temperatures tend to be equable with little marked seasonal variation and tend to vary with latitude.

Vegetation

The native vegetation of the tropical and sub-tropical islands, and of much of New Zealand, is generally forest, but there are areas of scrub and of reeds, and in New Zealand there are extensive areas of tussock grassland. The practice of shifting cultivation has had a profound effect on the vegetation of the tropical islands. This has resulted in areas of grassland and secondary forest in formerly forested areas. In New Zealand large areas of forest land have been converted to grazed pasture.

The soils have been grouped as far as possible into zones, primarily by latitude (Tropical; Sub-tropical; Temperate; Sub-polar; and Polar) and

partly by climate (Arid; Semi-arid; Sub-humid; Sub-humid to Humid; Humid, and Superhumid). The boundaries between adjacent zones are necessarily somewhat arbitrary.

Before considering the broad zonal soil differences, however, variation in the size and composition of microbial populations within a single locality is discussed with regard to seasonal and sampling differences and to the differences between forest land and grassland.

Later papers will deal with soils of the tropical and sub-tropical, temperate, sub-polar, and polar zones, and the respiratory activity of the soils.

METHODS

Sampling

The survey dealt with topsoil, and, where present, litter samples. At most sites, five separate soil samples of about 100 g each were collected along a transect at 10–20 yard (9–18 m) intervals. They were collected in sterile, screw-capped glass jars or in sterile polythene bags. They were brought to the laboratory as soon after collection as possible, and kept at 4°C. Microbiological examinations were made usually within a few days of their arrival in the laboratory.

For each sample the pH was determined electrometrically, and the moisture content was measured as loss of weight at 105°C and expressed as per cent wet weight. Conductivity measurements were made on 1:5 soil:water extracts of saline soils, and the total soluble salt concentration was expressed as milli-equivalents per cent (me. per 100 g) (Metson, 1956). For most of the New Zealand samples, detailed physical and chemical analyses of the soils were available. When they were not, per cent loss of weight on ignition at 600°C was measured to give some indication of organic content. Organic carbon may be assumed to be 0·6 x loss of weight on ignition, but for heavy clay soils, part of the loss of weight may be due to loss of bound water.

Dilution and Plating

A plating medium of pH 6·8 was prepared from 5 g glucose, 4 g Difco yeast extract, 5 g Difco Bacto-Tryptone, 1 litre distilled water, and 15 g agar. Ten grams from each sample were used to prepare a bulk sample of 50 g in 450 ml of sterile distilled water from which ten-fold dilutions were prepared. Duplicate plates were inoculated from dilutions 10^{-3} to 10^{-7}, and glucose broth tubes with Durham tubes and bromthymol blue as an indicator were inoculated from dilutions 10^{-1} to 10^{-7}. The dilutions were then pasteurised at 80°C for 5 minutes, and a second series of plates inoculated from dilutions of 10^{-2} to 10^{-6}. Plates and tubes were incubated at 24°C to 25°C.

Counts were made of the bacteria, actinomycetes, and moulds and of the bacterial spores germinating on the pasteurised plates. Growth and the production of acid and gas in the glucose broths were recorded. The presence or absence of distinctive chromogens and other types of bacteria forming readily recognised colonial forms, such as *Bacillus mycoides* (= *B. cereus* var. *mycoides*) was recorded.

Colonies of bacilli were isolated from the pasteurised plates, initially 10 isolates per soil, and subsequently 5, and slides were prepared from them. They were classified morphologically into the following groups: Group Ia, large Gram-positive rods with non-swollen sporangia, in which it is generally possible to distinguish *B. megaterium* from *B. cereus* and *B. mycoides*; Group Ib, small Gram-positive rods with non-swollen sporangia, which includes *B. pumilus* and *B. subtilis*; Group II, Gram-negative rods with swollen sporangia and oval spores, which includes *B. polymyxa* and *B. circulans*; and Group III, Gram-negative rods with drum-stick sporangia and spherical spores, containing *B. sphaericus* (Smith *et al.*, 1952). Colonies were also isolated from the unpasteurised plates for further study.

Respiratory measurements

The microbiological activity of the soils was determined by Warburg respiratory measurements (Umbreit *et al.*, 1949). These were carried out on a number of samples representative of the main soil groups occurring in each zone. Measurements were made on duplicate samples at 24°C, using approximately 3 g dry wt. of soil or approximately 0·2 g dry wt. of litter in each vessel. Four sets of measurements were made in each experiment: initial measurements of oxygen uptake and carbon dioxide production on the sample at field moisture on the morning of the first day of the experiment; subsequent measurements following the addition of 3 ml of distilled water on the afternoon of the same day; measurements under the same conditions the following morning; and final measurements after the addition of 0·5 ml of 1% glucose or asparagine, in the afternoon, to determine the respiratory response to fresh substrate. Bacteriological counts were made at the end of the experiment for a number of the earlier samples to determine whether there had been any marked change in population during the experiment.

POPULATION DIFFERENCES IN A SINGLE LOCALITY

Description of the Locality and the Sites Sampled

Variation in bacterial populations may be due to sample variation, seasonal variation, variation in soil properties or in vegetation, or to changes of population with time. These variations have been studied in

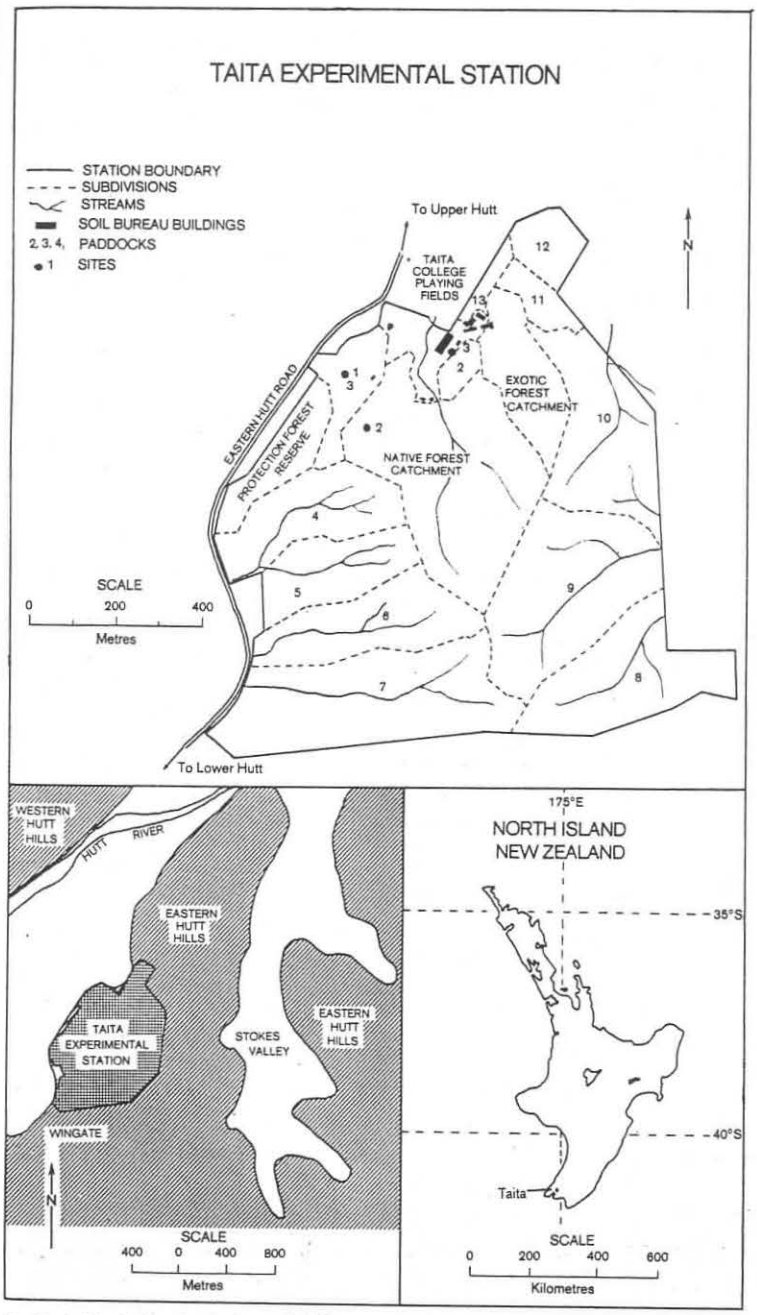

FIG. 1—Locality Map showing sampling sites:
Site 1. The main grassland site, near the Metereological Station, in Paddock 3.
Site 2. The beech forest site, in the Native Forest Catchment.
Site 3. The second grassland site, near Paddock 2.

the soils of the Taita Experimental Station (Fig. 1) at the southern end of North Island of New Zealand. A description of the area studied is given by Druce (1957) and by Atkinson (in press). The area is hilly with elevations rising to 720 ft (236 m) above sea level with considerable variation in slope, aspect, depth of profile, soil parent material (e.g. weathered rock, loess, or colluvium are present), moisture regime, and vegetation. The climate is temperate with a mean annual temperature of 54°F (12°C) and a mean annual rainfall of 54 in. (1372 mm). The soils show affinities both with the strongly weathered and strongly leached soils of the humid zone, and with soils of the sub-humid zone. The soils sampled were Taita hill soil and Taita clay loam. One catchment in the area is under native forest, while another is planted in exotic forest, and extensive areas are under grazed exotic pasture.

The differences imposed on the soil by vegetation are marked. There are profound differences between the canopies, the rooting systems, and the rate of organic turnover of forest land and grassland, and there are also differences in the fate of the dry matter produced. The established exotic pasture produces little dry matter, and since it is grazed, an appreciable part of the herbage is consumed by the grazing animals and, except for dung and urine, is not returned to the soil. The herbage remaining, and the dead root material, contribute therefore only about 1,500 lb/acre/yr (1,680 kg/ha/yr) as the net increment of soil organic matter. This organic increment is decomposed fairly readily, as is shown by the narrow C/N ratio (14) of the topsoil (Miller, 1962). In the beech forest, although an appreciable part of the dry matter produced each year is retained as new wood, almost all the dead leaves, twigs, and roots are returned to the soil, so that the annual increment, about 5,000 lb/ac/yr (15,600 kg/ha/yr), is much greater than in the pasture soil (Miller, 1963). The rate of breakdown is slower than in the grassland soil, measured by higher C/N ratio, which is 26 for the topsoil, and by the accumulation of organic litter layers in which turnover is about 3 years. Since the soils are assumed to be in equilibrium, however, the total decomposition must be greater in the beech soil.

There is a marked difference in the microclimates of forest land and grassland (Fig. 2). The soil under a forest canopy has smaller temperature and moisture fluctuations. The pasture topsoil is more subject to drying out than the beech forest topsoil.

The beech forest topsoil has more organic matter, as measured by loss on ignition, and is also more acid, pH 3·9 compared with pH 5·6 (Table 3, and Miller & Fitzpatrick, 1959).

Associated with these differences in soil chemistry, microclimate, and vegetation are differences in the animal populations (Miller *et al.*, 1955).

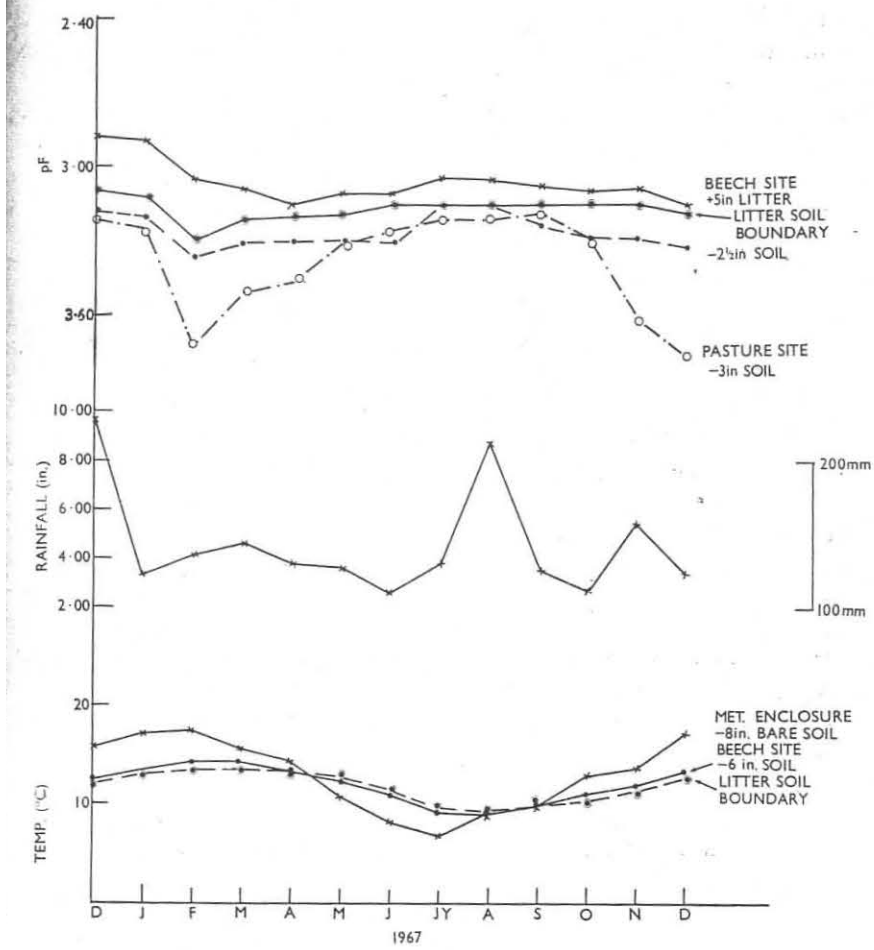

FIG. 2—Rainfall, soil moisture, and soil temperature readings for 1967.
Moisture readings at the beech forest and grassland sites.
Temperature readings at the beech forest and Metereological Station sites.

The forest fauna consists of a diverse population of litter animals; a topsoil fauna consisting chiefly of native earthworms, e.g. *Maoridrilus*; and a subsoil fauna principally of the native earthworm, *Octochaetus multiporus*. When forest is replaced with pasture, the litter disappears, and with it the litter fauna: the native topsoil earthworms disappear, and are ultimately replaced by introduced species, e.g. *Allolobophora caliginosa*. Only the subsoil earthworms of the original forest fauna persist, since only the subsoil environment is left substantially unchanged.

Parallel to these population differences are differences in the microfabric of the soil profile. The Taita clay loam is strongly weathered and has abundant mobile clay, not stabilised by flocculation. Under forest, the litter layer is a mixture of raw humus and a coarse type of silica-moder or mull-like moder and this microstructure continues into the upper layer of the topsoil, grading into a weak mull humus. The weak mull humus of the topsoil is inwashed along structure faces of the upper sub-soil which is a Braunlehm. Under pasture, this soil has a mull humus topsoil (Barratt, 1965).

Samples were collected from a number of sites on the Taita Experimental Station (Fig. 1). Two sites were examined in detail and a third (site 3) was also sampled. Site 1, a grassland site (Taita clay loam), had been initially covered by manuka (*Leptospermum scoparium*) scrub and gorse (*Ulex europeaus*), which had been felled, collected into heaps and then burned. The soil had subsequently been oversown with ryegrass and clover pasture and topdressed with superphosphate (Miller *et al.*, 1955; Roberts & Miller, 1956; Stout, 1961c; Miller & Fitzpatrick, 1959). This site had an elevation of 200 ft (61 m) and a 5° slope. Site 2 (Taita hill soil) was in the native forest vegetation catchment under hard beech (*Nothofagus truncata*), the climax vegetation under which the soil developed. The site had a slope of 20° and an elevation of 180 ft (59 m). Site 3 was a grassland site originally under native tussock grasses.

Because of changes occurring in the soil microflora of the grassland with time, it is necessary to know which part of the microflora is readily transported through the air, and consequently able to take advantage of changed ecological conditions in the soil. The airborne microflora was sampled with a slit-sampler three times at weekly intervals in November 1955, in an area adjacent to the buildings (Fig. 1).

RESULTS

Populations

Variation with Sample, Season, and Time in the Grassland Sites

Results of five separate samples and of two bulked composite samples from the principal grassland site (Site 1) are given in Table 1. Because of the effect of trail (Meiklejohn, 1957), the only valid basis of comparison of bacterial populations is between plates poured from the same level of dilution.

There was considerable variation between separate samples in total counts of bacteria, bacterial spores, actinomycetes, and moulds, which was least for the spores and greatest for the moulds. Variation was less between the composite samples. Populations of aerogenic fermenters and chromogenic bacteria showed greater variation than total counts. *Bacillus*

TABLE 1—Population Variation in Separate and Composite Samples of Topsoil taken from Site 1 under Introduced Pasture, collected October 1959

Soil Samples	Separate Samples 1	2	3	4	5	Mean	Composite Samples 6	7	Mean
Moisture (% wet wt)	35	25	49	26	51	37	—	—	—
Total bacterial count ($\times 10^6$/g wet wt)	1·5	1	4	0·5	1·5	1·7	·1·5	3	2·2
Spore count ($\times 10^5$/g wet wt)	3	3·5	6	8	2	4·5	5	1·5	3·25
Spores (%)	20	35	15	100	13	26	33	5	15
Actinomycetes ($\times 10^5$/g wet wt)	2·5	4	10	20	10	9·3	40	25	32·5
Moulds ($\times 10^4$/g wet wt)	25	2	20	30	2	16	50	15	32·5
Log. highest dilution positive									
Glucose broths. A&G	<3	3	4	4	4	3	3	4*	3
A	5	6	7	6	5	6	6	5	5
Orange chromogens	<3	6	3	3	3	3	<3	3	3
Chromobacterium lividum	<3	<3	<3	<3	<3	<3	<3	<3	<3
Pseudomonas fluorescens	<3	<3	<3	<3	3	<3	<3	<3	<3

* one of two tubes positive
A&G, acid and gas produced; A, acid produced

cereus or *B. mycoides* was present on the 10^{-3} spore plates from all dilution series and *B. megaterium* was also present in the two composite series. *Pseudomonas fluorescens* was present in one of the separate dilution series at a level of 10^{-3}, and this was the wettest. The occurrence of *Pseudomonas fluorescens* was the first record of this species on the Station after five years of sampling this site.

Subsequent samples taken from this site for respiratory measurements (Table 2) showed considerable seasonal variation; particularly a reduction in total bacterial and spore counts associated with very dry soil conditions in February and March 1962. *P. fluorescens* and *Chromobacterium* were present in the samples collected in November 1962, and *P. fluorescens* was also present in the samples collected in August 1963, but neither of these species was recorded from the other samples.

At Site 3, native tussocks (*Festuca* and *Poa*) had been planted on a bank cleared of topsoil and vegetation. The microflora was examined two years

after planting and consisted largely of orange chromogens, *B. cereus* and *B. mycoides* (Stout, 1960a). By March 1963, however, this soil was largely under exotic grasses and samples taken then contained *P. fluorescens*.

Variation with Season and Depth at the Beech Forest Site

The bacterial populations of the beech forest topsoil (Table 2) show far less seasonal variation than the grassland populations but far greater variation in the actinomycete population. Plate counts show that the mould population in the forest topsoil is greater than in the grassland topsoil, but seasonally as variable.

Mean populations of the different horizons of the beech profile are given in Table 3. Because the organic horizons have a density very different from

TABLE 2—Seasonal Variation of Populations (/g wet wt) and Respiratory Activity (μl CO_2/g LOI) in Composite Samples of Pasture and Beech Forest

	1962	1963				
	Spring November	Summer February	Autumn March/May	Winter August	Spring November	Means
Pasture Topsoils						
Moisture (% wet wt)	25	12	21	27	19	21
Loss on Ignition (% dry wt)	13	9	12	9	13	11
Populations						
Total count ($\times 10^6$)	8	0·2	0·6 2	5	2	3
Spore count ($\times 10^5$)	6·5	0·2	0·5 3	0·35	1	2
Spores (%)	8	10	8 15	0·7	5	6
Glucose broths A+G (log)	2	3	2 4	<1	3	2–3
A (log)	5	3	4 6	4	5	4–5
Actinomycetes ($\times 10^5$)	0·2	1·5	1 8	3	20	6
Moulds ($\times 10^4$)	1	3·5	4 4	3	2	3
Respiratory Rate at 24°C						
I. Initial unamended rate	30	26	n.d. 24	70	48	40
II. With water	13	71	29	34	49	39
III. ,, ,, (after 18 hr)	8	35	29	30	32	26
IV. With glucose	17	74	28	45	43	41
Beech Forest Topsoils						
Moisture (% wet wt)	34	26	26	45	26	31
Loss on Ignition (% dry wt)	26	40	27	27	29	30
Populations						
Total count ($\times 10^6$)	7	1	2 2	1	1	2·3
Spores ($\times 10^4$)	8·5	2·5	1·5 6	1·5	4	4
Spores (%)	1·2	2·5	0·75 3	1·5	4	2
Glucose broths A+G (log)	3	5	4 4	3	3	3–4
A (log)	5	6	4 5	5	3	4–5
Actinomycetes (log)	<3	<3	5 5	<3	5	?3
Moulds ($\times 10^5$)	0·5	11	15 4·5	8	10	8
Respiratory Rate at 24°C						
I. Initial unamended rate	42	33	n.d. 33	100	100	62
II. With water	17	48	46	30	99	48
III. ,, ,, (after 18 hr)	11	41	47	21	39	32
IV. With glucose	21	60	81	39	66	53

n.d. = not done
A+G, acid and gas produced; A, acid produced

nineral horizons, comparison of mineral and organic horizons eight basis is not valid, and therefore it is better to compare tions of the organic horizons and the mineral horizons For the organic horizons, the total bacterial population was ıer in the L layer than in the F or H layers, but populations of ermenters tended to increase with depth. The mould counts ıuch higher in the H layer. Spore populations were generally organic horizons, but orange and yellow chromogens were ous in some samples. In the mineral horizons, the highest were in the A horizon, and the populations in the B horizon ely low.

ith Vegetation

ns of the beech forest topsoil (A horizon) and the pasture ıle 3), showed little difference in total bacterial count, but the tinomycete populations were higher in the pasture topsoil, and ions of aerogenic fermenters and moulds were higher in the il. There was a marked contrast in the level of the actinomycete of the two sites and their numbers varied greatly in the beech ere was little difference in the *Bacillus* flora of the two sites *megaterium* tended to be the dominant species, but *B. circulans*

oil Properties, Mean Populations (/g wet wt), and Respiratory Activity organic carbon) in the Different Horizons of the Taita Hill Soil under Sampled in Five Successive Seasons, November 1962 to November 1963

	L	F	H	A	B_1	B_2
	7–3		3–0	0–3	3–10	10–18
	18–7·5		7·5–0	0–7·5	7·5–25	25–45
s						
	4·5		4·0	3·9	4·9	4·8
% wet wt)	30	55	57	32	20	20
ition						
t)	n.d.	n.d.	77	30	9	8
($\times 10^5$)	60	50	35	23	0·75	1·6
t ($\times 10^3$)	v	v	90	40	3·2	3
	—	—	3	2	4	2
oths A+G (log)	3	4·5	5·5	3·5	1	1
A (log)	6	5·5	6	4·5	4	3
tes ($\times 10^3$)	v	v	v	v	4·5	10
10^5)	v	v	20	8	0·2	0·07
tates at 24°C						
amended rate	140	200	200	140	150	180
er	270	220	90	90	70	75
(after 18 hr)	300	220	60	90	50	60
cose	450	500	80	100	70	75
aragine	350	390				

v, populations very variable
A+G, acid and gas produced; A, acid produced
n.d. (*cf.* Table 2) not done

TABLE 4—Morphological Classification of *Bacillus* Isolates (after Smith *et al.*, 1952)

		Morphological Groups					
		Ia		Ib	II	III	Total
		megaterium	*cereus/mycoides*				
Pasture Site	Topsoil	15	3	0	8	1	27
Beech Site	H horizon	10	6	2	10	1	29
	Topsoil	15	0	0	15	2	32
	Upper subsoil	9	5	2	3	0	19
	Lower subsoil	14	0	1	5	0	20
	Total	48	11	5	33	3	100

(Group II) was also important in both topsoils. *P. chlororaphis* has been recorded from the beech topsoil but not from the pasture, and conversely *P. fluorescens* has been recorded only from grassland sites.

Composition of the Airborne Flora

Altogether 105 ft^3 of air were sampled with the slit-sampler, and 2,000 bacterial and yeast colonies, and 7,000 moulds were cultured. The yeasts formed about 5% of the total bacterial and yeast colonies. Bacterial counts ranged from 3 to 64 ft^3, averaging 21 ft^3, and mould counts from 12 to 105 ft^3, averaging 31 ft^3 and there was no correlation between the levels of the two populations. A field immediately windward of the area was cultivated during the sampling period, but despite this, only about half the flora consisted of typical soil bacteria, and the rest were chromogens, which normally, with bacterial spores, constitute the airborne bacterial flora (Stout, 1960a; Gregory, 1961). Micrococci (24%) and other pigmented epiphytes (21%), constituted an important part of the flora. Soil bacteria were represented chiefly by spore-forming bacteria (39%) and by *Nocardia* or *Mycobacterium* (8%). There were no aerogenic fermenters amongst the isolates, nor did *Pseudomonas fluorescens*, *P. chlororaphis*, *Serratia* or *Chromobacterium* occur amongst the 2,000 colonies examined. Of 34 strains of spore-forming bacilli identified, the typical soil species, *B. megaterium* (9 isolates), *B. cereus* and *B. mycoides* (11) were the most common. Other isolates were *B. pumilus* (5), *B. subtilis* (4), *B. circulans* (1), *B. brevis* (1), and *B. sphaericus* (3).

Respiratory Measurements

Measurements of respiration rates are given in Tables 2 and 3. The beech samples without added water or glucose (Table 3) tended to have higher rates in the lower litter and H horizon than in the mineral horizons. There is little difference between the rates in the different horizons measured as CO_2 evolved/g organic C. Added water increased the rates in the litter horizons, which are subject to drying out, but depressed the rates in the other horizons. Only the two litter horizons showed a marked response to the addition of glucose or asparagine, there being a greater response with glucose.

The respiratory rate in the beech topsoil tended to be higher than in the pasture topsoil (Table 2), but seasonal fluctuations are similar for the two topsoils. The pasture samples were consistently drier than the forest samples, and the mean respiratory rate for the pasture samples immediately after the addition of water was the same as at field capacity, whereas the respiratory rate for the beech samples was depressed. Neither showed a very marked response to the addition of glucose.

The results for the topsoil samples collected in May 1963, are plotted in Fig. 3. The respiratory rate for the beech samples, showed a slight increase following the addition of water and a more marked increase after the addition of glucose, whereas there was no change in the rate for the pasture samples following the addition of water and only a slight increase following the addition of glucose. The graph also shows that the

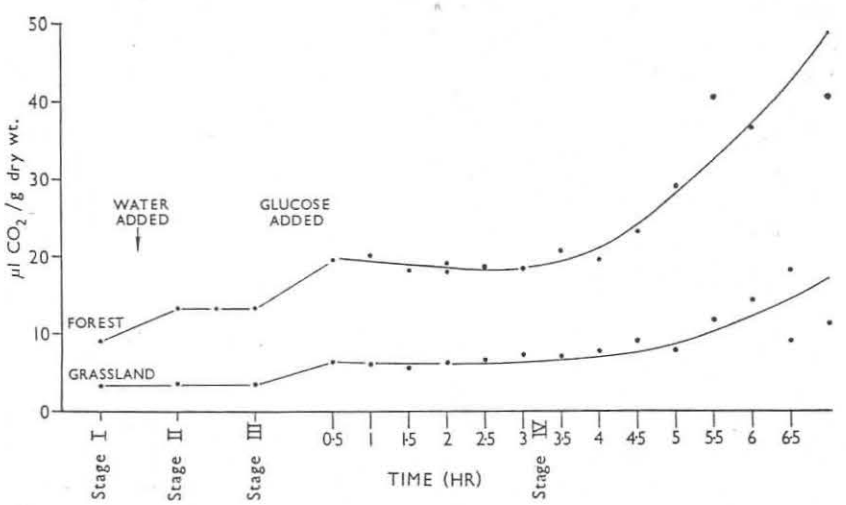

FIG. 3—Evolution of carbon dioxide at 24°C in grassland and forest topsoil samples collected in May 1963.

increase in respiratory rate after the addition of glucose may be considered in two parts: (i) an initial response, during which the rate is raised immediately, but then remains stable for about three hours; and (ii) a later response, relatively much greater, which takes place after the initial lag. This second response is no doubt associated with the proliferation of the microbial population (Drobník, 1960). Plate counts on samples three hours after the addition of glucose showed little difference from the original counts. It is evident therefore that respiratory measurements made over the first three hours after the addition of glucose record only the initial response before there is proliferation of the population.

DISCUSSION

Microbial populations under hard beech (*Nothofagus truncata*) are similar to those under manuka (*Leptospermum scoparium*) (Stout, 1961a), being greatest in the lower litter and topsoil horizons. *Enterobacter cloacae* is a notable species particularly in the H horizon. *Pseudomonas chlororaphis* is sporadically present, but not *P. fluorescens*, which occurred only in grassland samples, nor *Chromobacterium*, which occurred in the manuka litter and occasionally in grassland samples. *B. megaterium* tends to be more important than either *B. cereus* or *B. mycoides*. Under pine (*Pinus radiata*), populations are greatest in the F and H horizons (Stout, 1961a).

There appears to be a correlation between soil moisture and the occurrence of *P. fluorescens* which was recorded only from the wetter samples. Although the total counts are the least variable, populations of specific groups, such as the aerogenic bacteria (*Enterobacter*) or *P. fluorescens*, are a more sensitive index to ecological conditions.

There is continuous infiltration into the soil by the epiphytic microflora associated with falling leaves and leaf wash. This infiltration is similar to that by the airborne microflora. Both are distinct from the normal soil microflora. Desiccation and light, including U.V. irradiation, are the two principal factors limiting the survival of micro-organisms in the air, and to a lesser extent, on leaves. The survival of the yellow and orange chromogens is attributed to the role of protection against light of carotenoid pigments (Kunisawa & Stanier, 1958). Spores are also less subject to the adverse effects both of light and desiccation. Some data on the survival of different bacteria subjected to U.V. irradiation are given by Zelle & Hollander (1955) and are recorded in Table 5. These data probably explain why *P. fluorescens* and *Serratia*, which are relatively sensitive to U.V. irradiation, were not recorded in the airborne microflora, although they have been recorded from soils within 5 miles (9 km) of this site and on the windward side and also explain why a high proportion of the

TABLE 5—Effect of Radiation on Survival of Bacteria. Incident Energy at 2537 Å Necessary to Inhibit Colony Formation in 90% of the Organisms (after Zelle & Hollaender, 1955)

Species	Vegetative Cells	Spores
Pseudomonas aeruginosa	550	
P. fluorescens	350	
Proteus vulgaris	264	
Serratia marcescens	83–242	
Micrococcus candidus	605	
M. piltonensis	810	
M. sphaeroides	1,000	
Sarcina lutea	1,970	
Bacillus megaterium	113	273
B. subtilis	600–710	1200

airborne spores were *B. subtilis*, a relatively resistant species, although *B. megaterium* is the dominant species in the soil (Table 5). The frequent occurrence in the airborne flora of only a few groups of typical soil bacteria such as *Bacillus* and *Nocardia*, and the apparent absence of bacteria such as *Pseudomonas* or *Enterobacter* mean that if ecological conditions in a soil change, changes in populations will be initially restricted to species already present in the soil and colonization by other species may take some time, as appears to be the case with *P. fluorescens*.

It is clear that there are significant differences in the populations at different sites, associated particularly with differences in vegetation. These differences are related not only to the numbers in the main groups: bacteria, actinomycetes, and moulds, but also to the distribution of particular species, such as *P. fluorescens* and *Chromobacterium lividum*. In particular there is marked difference between forest or scrub sites, where there are well developed litter layers lying above the mineral soil, and pasture or grassland sites, where litter is absent. These differences over-ride the differences due to sampling or seasonal variation. This is also true of the soil fauna.

The Warburg measurements for the forest and grassland topsoils suggest that the rates are greater in the forest than in the grassland. This is not directly related to the moisture status of the soils, but rather to the fact that the higher moisture status of the forest soil favours a larger and more active population both of animals and of micro-organisms. Within the beech forest profile, the data suggest that the greatest respiratory activity is in the lower organic layers. The upper litter when dry has little respiratory activity, but when moist has a very high respiratory activity. The greater activity in the upper horizons of the beech forest profile is probably due to the favourable physical conditions for the fauna and microflora and the nutrients available in the freshly fallen plant debris.

These differences in the organic cycle in the type and quality of plant debris added to the soil, in the animal and microbial populations and their activity, are reflected in the character of the soil profile: in the differentation of horizons, their contrasting chemistry (pH, % organic C, C/N ratio) and in the association of mineral and organic matter forming the fabric of the soil itself. These are differences well illustrated in the present soils and in subsequent papers they will be examined in soils covering a wide range of climate, vegetation, and parent material.

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LITERATURE CITED

ATKINSON, I. A. E. (in press): Soil Survey of Taita Experimental Station. *New Zealand Soil Bureau Bulletin 32.*

BARRATT, B. C. 1965: Micromorphology of Some Yellow-Brown Earths and Podzols of New Zealand. *New Zealand Journal of Agricultural Research 8:* 997–1042.

DROBNÍK, J. 1960: Primary Oxidation of Organic Matter in the Soil. 1. The Form of Respiration Curves with Glucose as Substrate. *Plant and Soil 12 (3):* 199–211.

DRUCE, A. P. 1957: Botanical Survey of an Experimental Catchment, Taita, New Zealand. *New Zealand Department of Scientific and Industrial Research Bulletin 124:* 1–81.

EDWARDS, P. R.; FIFE, Mary A. 1955: Studies on the *Klebsiella–Aerobacter* Group of Bacteria. *Journal of Bacteriology 70:* 382–390.

GREGORY, P. H. 1961: "The Microbiology of the Atmosphere". Leonard Hill, London. 251 pp.

KUNISAWA, R.; STANIER, R. Y. 1958: Studies on the Role of Carotenoid Pigments in a Chemoheterotrophic Bacterium. *Archiv für Mikrobiologie 31:* 146–156.

MEIKLEJOHN, Jane, 1957: Numbers of Bacteria and Actinomycetes in a Kenya Soil. *Journal of Soil Science 8:* 240–247.

METSON, A. J. 1956: Methods of Chemical Analysis for Soil Survey Samples. *New Zealand Soil Bureau Bulletin 12:* 1–208.

MILLER, R. B. 1962: Biological and Chemical Changes following Scrub Burning on a New Zealand Hill Soil. 4. Changes in Some Chemical Properties. *New Zealand Journal of Science 5:* 259–68.

—— 1963: Plant Nutrients in Hard Beech. III. The Cycle of Nutrients. *New Zealand Journal of Science 6:* 388–413.

MILLER, R. B.; FITZPATRICK, M. 1959: Biological and Chemical Changes Following Scrub Burning on a New Zealand Hill Soil. 2. Changes in Soil pH. *New Zealand Journal of Science 2:* 171–181.

MILLER, R. B.; STOUT, J. D.; LEE, K. E. 1955: Biological and Chemical Changes Following Scrub Burning on a New Zealand Hill Soil. *New Zealand Journal of Science and Technology B37:* 290–313.

ROBERTS, H. S.; MILLER, R. B. 1956: A Statistical Design for Fertiliser Experiments on Variable Hill Soils. *New Zealand Journal of Science and Technology B38:* 112–119.

SMITH, N. R.; GORDON, R. E.; CLARKE, F. E. 1952: Aerobic Spore-Forming Bacteria. *U.S. Department of Agriculture, Agricultural Monograph 16:* 1–148.

SNEATH, P. H. A. 1960: A Study of the Genus *Chromobacterium. Iowa State College Journal of Science 34:* 243–500.

STANIER, R. Y.; PALLERONI, N. J.; DOUDOROFF, M. 1966: The Aerobic Pseudomonads: A Taxonomic Study. *Journal of General Microbiology 43:* 159–271.

STOUT, J. D. 1958: Biological Studies of Some Tussock Grasslands. IV. Bacteria. *New Zealand Journal of Agricultural Research 1:* 943–957.
—— 1960a: Biological Studies of Some Tussock Grasslands. XV. Bacteria of Two Cultivated Soils. *Ibid. 3:* 214–223.
—— 1960b: Bacteria of Soil and Pasture Leaves at Claudelands Showgrounds. *Ibid. 3:* 413–430.
——1961a: A Bacterial Survey of Some New Zealand Forest Lands, Grasslands and Peats. *Ibid. 4:* 1–30.
—— 1961b: The Antibiotic Relationships of Some Free-Living Bacteria. *Journal of General Microbiology 27:* 209–219.
—— 1961c: Biological and Chemical Changes Following Scrub Burning on a New Zealand Hill Soil. 3. Microbiological Changes. *New Zealand Journal of Science 4:* 740–752.
—— 1964: Bacterial Populations of Some Grazed Pastures in Hawke's Bay. *New Zealand Journal of Agricultural Research 7:* 91–117.
UMBREIT, W. W.; BURRIS, R. H.; STAUFFER, J. F. 1949: "Manometric Techniques and Tissue Metabolism". 2nd ed. Burgess Publishing Co., Minneapolis. 227 pp.
ZELLE, M. R.; HOLLAENDER, A. 1955: Effects of Radiation of Bacteria. Pp. 365–430 *in* "Radiation Biology", Vol. II. (Ed. A. Hollaender). McGraw-Hill, New York.